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Gene Transfer Safety Symposium:

Current Perspectives on Gene Transfer for X-SCID

NIH Recombinant DNA Advisory Committee Meeting

March 15, 2005

Bethesda Marriott
Bethesda, Maryland

SUMMARY

Background

On March 15, 2005, the National Institutes of Health's (NIH) Office of Biotechnology Activities (OBA), convened the safety symposium "Current Perspectives on Gene Transfer for X-SCID" to examine the safety issues related to the use of gene transfer in clinical trials for X-linked severe combined immunodeficiency disease (X-SCID).

The goals of this symposium were to promote scientific understanding and public awareness of the latest research findings regarding treatments for SCID as well as retroviral integration and insertional mutagenesis. The symposium was divided into three sessions. The first session provided a comprehensive overview of current U.S. and international trials investigating gene transfer as a possible treatment for SCID, the second session focused on the latest research into retroviral integration and mechanisms of insertional mutagenesis, and the third session explored the use of bone marrow and stem-cell transplantation (SCT) as an alternative treatment for SCID.

Specifically, this symposium sought to explore answers to the following questions:

- Should the assessment of the balance of potential benefits and risks in X-SCID protocols be modified in light of the leukemia cases in the French X-SCID trial?
- Does it remain prudent and medically defensible for the NIH to recommend limiting X-SCID retroviral gene transfer to individuals who have failed identical or haploidentical SCT or for whom no suitable stem-cell donor can be identified?
- Should the assessment of the balance of potential benefits and risks in other SCID protocols be modified in light of the French leukemia cases?
- Should the assessment of the balance of potential benefits and risks in protocols for other disease indications using retroviral vectors be modified in light of the leukemia cases?

- What new information should be communicated to participants in ongoing protocols, prospective participants in new protocols, and participants who participated in retroviral vector trials closed to further enrollment or past the protocol-defined followup period?

- Are there additional questions about preclinical research that should be developed as points to consider for investigators preparing protocols involving retroviral vector gene transfer?

- How might the risk of leukemia be reduced in gene transfer studies using retroviral vectors?

- What is the ethical status of a research intervention that provides effective therapy but carries a severe risk? Should such an intervention be considered "therapy" if some or all of the participants develop serious adverse events (SAEs) albeit after years of normalized life?

Background of X-SCID Safety Issues: RAC Conclusions and Recommendations

On December 5, 2002, and February 10, 2003, the RAC reviewed the clinical and molecular data concerning two AEs that occurred in a human gene transfer study being conducted in France to correct X-SCID. This study involves engraftment of an autologous bone marrow-derived, CD34+ hematopoietic stem-cell enriched cell population transduced with a Moloney murine leukemia retrovirus-derived replication incompetent vector encoding the common gamma chain ((c) transmembrane protein subunit shared by receptors for Interleukins 2, 4, 7, 9, 15, and 21. Two children in this study developed T-cell acute lymphoblastic leukemia almost 3 years after receiving the gene transfer. The leukemias in both children appear to share the common causative mechanism of insertional mutagenesis at or near the *LMO-2* gene with the aberrant production of LMO-2 protein, which contributed to the abnormal growth of these leukemic cells. An analysis of the available data from this and other gene transfer clinical trials for SCID led the RAC to conclude that:

- The majority of children in this X-SCID gene transfer study have had major clinical improvement to date.

- Of the nine children in this experimental study who had successful engraftment of their (c-transduced cells, two developed leukemia approximately 3 years after treatment and have required chemotherapy; the overall frequency of this AE in this trial cannot be determined at this time.

- The gene transfer was a cause of both leukemias.

- The occurrence of leukemia in this protocol is not a random event and constitutes a serious inherent risk in this study.

- Some participants in gene transfer studies for non-X-SCID experienced mild to moderate clinical improvement.

These findings led the RAC to make the following recommendations, which will be reviewed and potentially revised as new data become available:

- Pending further data or extenuating circumstances, reviewed on a case-by-case basis, retroviral gene transfer studies for X-SCID should be limited to participants who have failed identical or haploidentical SCT or for whom no suitable stem-cell donor can be identified. Case-by-case review would include appropriate risk:benefit analysis accompanied by implementation of appropriate informed consent and monitoring plans.

- There are neither sufficient data nor reports of AEs directly attributable to the use of retroviral vectors at this time to warrant cessation of other retroviral human gene transfer studies, including

studies for non-X-SCID. Such studies may be justified contingent on an appropriate risk:benefit analysis accompanied by implementation of appropriate informed consent and monitoring plans.

Opening Remarks

Amy P. Patterson, M.D., Director, OBA, NIH, set the stage for this symposium by reviewing the goals, the agenda, and a short history of the RAC's involvement in these issues.

Diane W. Wara, M.D., University of California, San Francisco, reiterated the goal of this safety symposium, which was to carefully revisit the statement and recommendations put forward by the RAC in February 2003 regarding gene transfer for X-SCID. As a pediatric immunologist, she has provided care for children with SCID and was thrilled at the reports of successful immune reconstitution by gene transfer in the French trial. Now with the occurrences of leukemia among participants in that trial, the dilemma faced by both the scientific community and care providers is to consider how to proceed with gene transfer vs. the standard of care, BMT, for SCID patients. In light of new information, the role of the RAC will be either to endorse or revise the February 2003 statement.

Theodore Friedmann, M.D., University of California, San Diego, stated that this safety symposium was an opportunity for the RAC members to examine the issues surrounding the problems in the French X-SCID study and to develop recommendations for investigators in the field and other interested parties. Gene transfer research is at a very early, immature stage; the technology is at a nascent period of development, and the techniques are still unrefined and carry enormous risk. However, the French X-SCID gene transfer study has provided the first strong proof of principle for the therapeutic efficacy of gene transfer. The same risk:benefit analysis should be applied to gene transfer as to other experimental procedures. With the French X-SCID study, the gene transfer field has had the opportunity, for the first time, to calculate an actual risk:benefit ratio. The field should focus more research on overcoming the scientific and medical obstacles.

As of winter 2005, of the 18 participants in the French (12 participants) and British (6 participants) trials, 17 experienced immune reconstitutions, and 1 did not respond. Three participants experienced SAEs, including the death of one child from leukemia. The other two children have received chemotherapy and are currently in remission. Severe risks have arisen in the context of a robust clinical therapeutic effect. The risk of leukemia is inherent in the biology of the vector system. The integration event itself is responsible for the SAE, and until integration mechanisms are manipulatable, that is unlikely to change. The clinical dilemma is what to do with individuals who require treatment before improved technology is developed. These issues are not new in clinical medicine. Historical examples of treatments that have gone through a similar process during development include treatment of childhood lymphocytic leukemia and Hodgkin's disease, organ transplantation, and monoclonal antibody development.

The RAC discussion should focus on determining the criteria for proceeding with current studies, and the definition of long term research goals to improve the technology and increase safety. The task before the RAC is to help assist investigators to ensure high quality gene transfer clinical studies and maximal safety for research participants. Dr. Friedmann also enumerated the specific end points for the RAC's consideration during this symposium's discussion:

- Affirm or modify the February 2003 RAC position on retrovirus-mediated gene transfer for X-SCID and other SCIDs and discuss whether any of that thinking should be extended to other stable transducing systems such as herpesvirus and adenovirus.

- Anticipate the effect of additional SAEs in the French X-SCID study.

- Develop preclinical questions, in addition to those included in *Appendix M,* to assist investigators with such issues as determining the minimal number of transduction events and transduced cells necessary for therapeutic effect, elements to regulate levels of gene expression, methods to

ablate genetically modified cells and reverse their effects, and methods to test and archive grafted cells to detect inappropriate selection and/or expansion.

Session I: Current SCID Gene Transfer Experience

Moderators: Bernard Lo, M.D., University of California, San Francisco, and Diane W. Wara, M.D., University of California, San Francisco

Approaches to the Patient with X-Linked SCID Who Has Failed Standard Bone Marrow Transplantation (BMT) Treatment

Jennifer M. Puck, M.D., National Human Genome Research Institute (NHGRI), NIH, provided a brief introduction to X-SCID, noting that the incidence of X-SCID is approximately 1 in every 50,000 to 100,000 live births. The definition of this syndrome is a profound lack of both T and B cell immunity. Patients suffer excessive recurrent and opportunistic infections early in life when maternal antibodies wane. These infants then develop failure to thrive, a condition that is fatal in infancy unless an immune system can be provided. Only males are affected by this condition because they have one X chromosome; females who are carriers are unaffected.

Dr. Puck is head of the molecular diagnostics mutation detection lab that analyzes SCID samples. The molecular cause of SCID can be diagnosed in the majority of patients today, which enables carrier and prenatal diagnosis in families who have been previously identified, may help predict responses and tailor BMT, and is a prerequisite for gene transfer. There are multiple types of SCID caused by mutations in different genes. X-SCID accounts for approximately 50% of SCID cases. While there are different mutations that cause X-SCID, there is little clinical diversity with most patients severely affected.

SCID is treatable by BMT, with the best results obtained if the bone marrow is transplanted early in life, before serious infections occur. Best results are obtained if a patient has a sibling donor who is matched for human leukocyte antigen (HLA). Even without an HLA-matched sibling, some patients may be completely or substantially cured by BMT. However, many patients require long-term immunoglobulin replacement after BMT, and some are only partially immune constituted.

X-SCID was a candidate for gene transfer because of the limitations of BMT, the gene is expressed in all blood lineages and is not tightly regulated. The transduced cells would have a selective advantage. Because the immune system is lacking, an immune response to the vector or transduced cells is not an obstacle as commonly occurs in other gene transfer applications.

The NIH Clinical Trial of Gene Therapy for X-SCID is a salvage treatment protocol. Investigators have selected children who, despite repeated BMT, have unsatisfactory immune reconstitution. These research participants are ages 6 to 19, have experienced one to four BMT attempts, have had growth failure, and suffer from a variety of chronic conditions. Some of these children are on intravenous nutrition because they do not eat properly. Some receive gastrostomy feedings. Most have chronic skin conditions, and several have fibrosis of the lungs. The oldest participant is a victim of cancer—hepatocellular carcinoma possibly related to an unidentified virus.

In these patients, Dr. Puck explained that an analysis of T-cell receptor excision circles compared with adult controls shows that the levels of new CD8 and CD4 cytotoxic and helper phenotype cells being produced in the thymus are extremely low or undetectable. This situation indicates that any BMT/SCT that has occurred has passed its usefulness and that there is either no or very low production of new T cells. However, CD34+ cells are present so that gene transfer could be an option for these patients.

Preliminary Results of an NIH Trial of Retroviral Interleukin-2 Receptor Gamma (*IL2RG*) Gene Transfer as Salvage Treatment for Older Post-BMT SCID Patients

Harry L. Malech, M.D., National Institute of Allergy and Infectious Diseases, NIH, described the preliminary results of the NIH trial of retroviral *IL2RG* receptor gene transfer as salvage treatment for older post-BMT SCID patients. This protocol, "Ex vivo retroviral gene transfer for treatment of x-linked severe combined immunodeficiency" was designed to be salvage therapy for individuals without an HLA-matched sibling who continue to have clinically significant immune function impairment despite previous BMT from a haploidentical donor. The protocol would enroll participants from 2-20 yrs in age, who had previous BMT, but no/low engraftment and antigen/mitogen response. The participants would be IVIG dependent, and have infections, and chronic disease.

Two participants have been enrolled. P1 has variant SCID with a polyA mutation that allows some γc expression, enough to have rejected BMT. P2 has conventional SCID, and has very low T cells from a maternal BMT. For P1, at 6 months posttransfer, about 4.3 percent of his CD3 cells were positive for vector, and at 12 months vector was found in about 2.6 percent of his CD3 cells. This individual experienced improved well-being, fewer missed school days, and resolution of some chronic abdominal pain. After a growth plateau, he grew 2 centimeters in 6 months. New cervical lymph nodes and tonsils appeared, abdominal distention decreased, and eczema improved. P2, currently at 6 months posttransfer, is showing a vector copy number of 1.37, which suggests that most of his circulating T cells and some of his B cells are positive for vector. This individual had suffered from headaches and was suffering from fewer headaches at 6 months, had reduced frequency of daily diarrhea, and had a significant growth in height and in weight.

To date, there were no adverse effects in the participants. Postinfusion peripheral blood shows polyclonality of the transduced cells, and replication-competent retroviral (RCR) testing was negative in both the products and the patient cells. In terms of efficacy, there is multilineage marking of blood cells, with evidence of selective marking of T cells to a modest extent in one participant and robustly in the other. In P2 at 6 months, CD4 T cells increased, naive T cells increased, and there was a new and substantial T-cell proliferative response, particularly to candida.

Retroviral Gene Transfer for Treatment of X-SCID and Adenosine Deaminase (ADA)-SCID

Fabio Candotti, M.D., NHGRI, NIH, presented results from the London X-SCID trial conducted by Dr. Ian Thrasher that is similar to the French trial. The vectors are similar; however, the vector used in the London trial is packaged using the Gibbon ape leukemia viral (GALV) envelope and serum-free culture conditions were used. The pattern of reconstitution in the first seven participants shows that during the 20 to 40 weeks after gene transfer there is an increase in CD3+, CD4+, and CD8+ T cells. Three participants are more than 30 months post gene transfer, the problematic period in the French trial. Four of the participants are off prophylaxis.

Dr. Candotti also discussed adenosine deaminase (ADA) deficiency, another form of SCID (ADA-SCID), which accounts for between 15 and 20 percent of all SCIDs. It is autosomal recessive, so it affects both males and females. In addition to having an immune deficiency, ADA-deficient individuals also suffer from effects on lungs, liver, skeletal system, and central nervous system. ADA-SCID is a global disease that can present as a severe form with a classical presentation of immunodeficiencies, early onset, and catastrophic possibility for infection or with a more delayed and milder form diagnosed later in life. Conventional treatment for ADA deficiency is based on allogeneic BMT and originally on hematopoietic SCT. HLA-identical sibling donor transplantation is very successful in the survival of these patients, but the results of parental transplantation are generally poor. Many ADA-SCID patients are treated with enzyme replacement therapy with bovine ADA enzyme, which is conjugated to polyethylene glycol (PEG-ADA) to increase stability and reduce immunogenicity. However, these patients rarely experience a correction of function, and all develop antibody against the bovine ADA as soon as their immune system improves.

Dr. Candotti summarized the history of the nine clinical trials that have been conducted for ADA-SCID in the US, UK, Japan, Italy, and the Netherlands. In those trials, 25 ADA-SCID individuals have been treated using retroviral gene transfer into T cells in the earliest trials or HSC in the majority of the trials. Improved immune function was first achieved in the participants in an Italian trial, which introduced nonmyeloablative conditioning before gene transfer in the absence of PEG-ADA. Three of these participants received gene transfer more than 30 months ago, thus are beyond the period in which the leukemias developed in the X-SCID participants. In total, 22 participants have been dosed using gene transfer into stem cells; 19 were infants or children, 2 were adolescents, and 1 was an adult. Eight of the individuals with detectable gene marking are alive and have not experienced AEs. The available data on the vector insertion does not show a difference between the patterns of ADA vector integration compared with X-SCID vector integration. The solid safety record may indicate that because ADA-SCID is a different disease, the risks involved with gene transfer are different from those associated with X-SCID.

Summary of Recent Safety Data Emerging from the French X-SCID Trial

Theodore Friedmann, M.D., University of California, San Diego, presented an update on the French X-SCID trial with information received from Dr. Fischer and Agence Francaise de Securite Sanitaire des Produits de Sante (AFSSAPS). Participant #4, who experienced the first SAE, was treated at age 1 month with a dose of 18 x 10^6/kg cells. Clinical leukemia occurred 30 months later. He was responded to chemotherapy and BMT, but relapsed and died 5 years after gene transfer (in October 2004). The vector had integrated into the first intron of LMO-2 resulting in elevated LMO-2 expression. This individual also had a family history of cancer and a partial trisomy at t(6;13).

Participant #5 received gene transfer at age 3 months with a dose of 20 x 10^6/kg cells, experienced clinical leukemia at 34 months of age, was treated, and is currently alive and well. Three leukemic mature T lymphocytes were detected with the same single integration upstream of LMO-2.

Following these AEs, the protocol was modified so that participants now must be older than 6 months of age, and there must be no family history of cancer and no cytogenetic abnormality. The dose was reduced to less than 10 x 10^6/kg cells.

The third leukemia SAE developed in participant #10, who received gene transfer at nine months with a dose of 11 x 10^6/kg cells. In January 2005, 33 months post-gene transfer, this individual developed rhinopharyngitis with progressive cervical adenopathy, wheezing, and respiratory distress from an enlarged mediastinum. This child was found to have a single proliferated immature cortical thymocyte clone and blast cells in the blood, marrow, and nodes. The leukemic cells in this child contained multiple integrated proviruses; however, the integration sites were not yet identified. This child shows no cytogenetic abnormality in proliferating cells, has responded rapidly and well to corticosteroid treatment, and is currently in complete remission.

Dr. Friedmann noted that the current thinking of Dr. Alain Fischer, principal investigator of the French X-SCID trial, is that the French trial will pursue development of a self-inactivated (SIN) lentiviral system rather than continuing to use the Moloney leukemia-based vectors. The trial currently remains on clinical hold.

U.S. Food and Drug Administration (FDA) Perspectives

Carolyn A. Wilson, Ph.D., FDA, reviewed FDA actions in January 2003, the administrative and investigational new drug (IND) status of 28 INDs on clinical hold since January 2003, related FDA actions in January 2005, and the March 2005 meeting of the Cellular, Tissue, and Gene Therapies Advisory Committee (CTGTAC).

In January 2003 the FDA sent three different letters to sponsors of IND trials using retroviral vectors. The first letter was sent to sponsors that had active INDs that used *ex vivo* transduction with retroviral vectors

of hematopoietic stem cells. This included the SCID INDs as well as any other clinical indication that uses this method. These sponsors received a letter placing them on clinical hold until they were able to revise their informed consent documents and develop methods to monitor the clonality of vector integration. The FDA sent the second letter to sponsors with inactive INDs but using the same approach in terms of *ex vivo* transduction; they were notified that if they were ever to resume a clinical trial under that IND, they would need to satisfy the same requirements as similar trials currently in active status. All other retroviral vector clinical trial sponsors that used target cells other than hematopoietic stem cells received a third FDA letter informing them of the French X-SCID events and recommending that they revise their informed consent documents and develop methods to monitor clonality; these trials were not put on clinical hold. In addition, all sponsors were asked to provide a risk:benefit analysis, and the FDA has evaluated each response.

In response to the recent developments in the French X-SCID trial, in January 2005 the FDA placed three IND trials on clinical hold—two for X-SCID and one for ADA-SCID. The letter the FDA sent to these sponsors requested that they revise their informed consent documents and notify their institutional review boards (IRBs) and also advised them that the FDA would be convening the CTGTAC to discuss these events. The FDA also sent a letter to all sponsors of retroviral vector trials to inform them of the new events and notified IRBs that regulate retroviral vector clinical trials to ensure that they were aware of these new events.

In early March 2005 the FDA convened a meeting of the CTGTAC (formerly the Biological Response Modifiers Advisory Committee). This meeting began with a review of the mechanisms of retroviral insertional mutagenesis. The CTGTAC was updated on what had happened in the French X-SCID trial and the regulatory actions that had been taken. FDA representatives reviewed what had happened at the CTGTAC meeting in February 2003, the recommendations that resulted from that meeting, and what subsequent actions had been taken in response to those recommendations. A more detailed update on the events in France also was provided. In addition, three speakers provided updates from nonhuman animal models. In answer to questions posed to the CTGTAC by the FDA, recommendations from the CTGTAC included the following:

- Until sufficient data accumulate to change the risk:benefit assessment in a more favorable manner, retroviral vector-mediated gene transfer should be used only in children with X-SCID under the following conditions: those who have failed previous hematopoietic SCT or BMT or those who have no reasonable alternative therapies.

- The CTGTAC advised against considering vector copy number or number of transduced cells to address the risks in gene transfer for X-SCID but strongly encouraged investigating alternative approaches, including new retroviral vector products to lessen risk. The CTGTAC noted that it considered suicide vector systems feasible, but in all cases the CTGTAC asked that adequate testing in relevant nonhuman animal models be performed to validate any novel approach.

- In the case of ADA-SCID, the CTGTAC recommended that these trials be allowed to proceed. They stated clearly that the risks are still present and that investigators and participants should be informed of these risks using strong and clear communication. The CTGTAC also noted that if a retroviral vector-related malignancy were to develop in the future in any ADA-SCID clinical trial, they would ask the FDA to reconvene the CTGTAC to reassess these risks. In other non-SCID clinical indications, the CTGTAC recommended allowing clinical trials to proceed but acknowledged that risks remain present.

- The CTGTAC did not recommend a specific allowable vector copy number per cell but acknowledged that copy number is an important issue. They suggested that each IND be assessed on the basis of available data and that nonhuman animal models be used by investigators to assess the relative risk of leukemia induction with increased copy number.

Discussion

A discussion followed comparing the third AE to the previous two in the French X-SCID trial. The discussion was limited because information about the insertional mutations was not yet available. In response to the earlier leukemias, the investigators had modified the protocol to limit enrollment to participants older than three months of age, and lowered the dose of transduced CD34+ cells; however, these additional safety precautions did not appear to minimize risk adequately. In determining dose, it is necessary to consider both efficacy and safety. Lowering the dose of transduced cells would be expected to lower risk by decreasing the probability of integration into a gene that cooperates in oncogenesis. However, a CD34+ threshold effect may exist in which too few cells may not properly engraft. Limiting the number of stem cells may stress them to divide more frequently, leading to increased probability of spontaneous mutations in oncogenes. More data, possibly from animal models, is needed to determine sufficient doses in specific situations.

Session II: Retroviral Integration and Insertional Mutagenesis

Moderators: Marina O'Reilly, Ph.D., Office of Biotechnology Activities, NIH, and Naomi Rosenberg, Ph.D., Tufts University

Gene Transfer and Insertional Mutagenesis

Christof von Kalle, M.D., Cincinnati Children's Hospital Medical Center, reviewed the use of LAM-PCR to study retroviral vector integration sites and the development of clonality. He presented data from research participants 4 and 5 in the French X-SCID trial that demonstrated the progression from polyclonality to oligoclonality to a monoclonal lymphoproliferation. The insertion of the retroviral vector near an oncogene, LMO-2, along with expression of the γc transgene may have contributed to the clonal proliferation that then developed into leukemia after the acquisition of additional mutations.

Murine leukemia virus (MLV)-based vectors prefer to integrate in genes with 59 percent of integrations within or close to genes and 39 percent within the gene itself, with about 50 percent of these occurring within the first 20 percent of the gene. Insertion peaks around the start of transcription (+/- 5 kb). Clustering was observed in common insertion sites often in genes with kinase or transferase activity. Differences are observed before and after engraftment in the distribution of insertion sites. Differences between trials and trial subpopulations remain to be interpreted. For example, insertion in the vicinity of LMO-2 occurred relatively frequently in the French X-SCID data but was not detected in the British X-SCID population.

Dr. von Kalle also described the results of transient transfection experiments to analyze how the MLV vector activates LMO-2 expression. LMO-2 activation appeared to be affected by the enhancer of the 3' LTR. Significant activation was not detected with the 5' LTR possibly due to promoter competition or the presence of negative regulatory elements upstream of the LMO-2 promoter. SIN vectors in which the 3' LTR was deleted prevented LMO-2 promoter activation suggesting that SIN vectors may be advantageous for gene transfer; however, it is difficult to produce large scale volumes of SIN retroviral vectors with sufficient titers.

Gene Transfer Insertional Mutagenesis Insights

Neal G. Copeland, Ph.D., National Cancer Institute, NIH, described the use of LAM-PCR and the mouse genome sequence to analyze retrovirus insertion sites in mouse tumor cells and the development of the retroviral tagged cancer gene database (RTCGD). So far 482 candidate cancer genes have been identified by insertional mutagenesis, which represents approximately 2% of the mouse genome. Cancer appears to result from mutations in multiple genes that cooperate to induce disease. The mouse tumor cells frequently have from two to ten independent integrations suggesting that the process of oncogenesis may involve integration, insertional mutagenesis which provides the cell with a selective advantage, clonal expansion, followed by further insertions in cooperating genes until cancer occurs.

A search of the RTCGD revealed five tumors with integrations in LMO-2, three tumors with insertions in IL-2 RG (the γc transgene in the X-SCID vector), of which two tumors had insertions in both LMO-2 and IL-2 RG. This suggested that *LMO2* and *IL2RG* cooperate to induce leukemia in SCID patients and that *IL2RG* provides the first oncogenic hit, whereas *LMO2* provides the second hit. These findings may bode well for gene transfer trials involving other genes that are not likely be oncogenic when expressed in a retrovirus. To study this, mice were infected with a retroviral vector expressing *sox4*, a known oncogene, and leukemic cells were analyzed for common insertion sites to identify genes that cooperate with the oncogenic transgene.

To begin to answer the question of why all the participants in the French X-SCID trial did not develop T-cell leukemia, Dr. Copeland and colleagues investigated the number of additional mutations it might take to induce a tumor in the cells with integrations at *LMO2* or *IL2RG* or both genes. As many integration sites as possible were identified in the leukemias and 47% of integrations occurred in candidate cancer genes in the RTCGD. The data suggested that many additional mutations were necessary to induce tumorigenesis in the cells with LMO-2 and IL-2 RG insertions. Dr. Copeland suggested changes to the X-SCID protocol that might lower risk including transplanting fewer transduced cells and using SIN vectors and an endogenous *IL2RG* promoter.

Retroviral Integration Target Site Preferences

Frederic D. Bushman, Ph.D., University of Pennsylvania, described the genome-wide survey of integration targeting by HIV, MLV, and avian sarcoma leucosis virus (ASLV). HIV favors integration into active genes. MLV favors integration near transcription start sites and CpG islands. ASLV has a weaker preference for integration into genes which may beneficial in gene transfer vectors, however, random integration would still occur in genes in 35% of events. The preference for active genes may be due to exposure of DNA in open chromatin or active transcription units. However, the differences between HIV and MLV integration suggest a more complex mechanism. There may be specific interactions between integration complexes and locally bound proteins on target DNA.

Regarding targeting in other integration systems, Dr. Bushman stated his preference for using retroviruses and lentiviruses. Adeno-associated virus and LINE elements seem to rearrange the DNA at their integration sites. Transposons create double-strand DNA breaks. Homologous recombination would be ideal, but improving efficiency is a major challenge, and a solution to that dilemma is not likely in the near future.

Dr. Bushman reviewed the possible strategies for controlling integration targeting. Studies are ongoing to identify both the viral determinants of integration by creating chimeras of viruses with different integration preferences and the cellular determinants by knocking out or down candidate factors in cells. Dr. Bushman expressed his optimism that progress will occur soon. In *in vitro* systems, it has been possible to direct targeting by fusing integrase to specific DNA binding proteins. Work in the field is focusing on modifying targeting by swapping viral integration systems or mutating integrase to remove undesirable or create favorable tethering interactions.

Discussion

The participants and presenters discussed the factors that may contribute to the potential for insertional mutagenesis, and what modifications may lower that risk such as dosage, vector design, target cell, transgene expression, and host factors. Vectors with potential safety modifications will need to be directly compared in animal models. The FDA and National Toxicology program are collaborating to design large animal studies to compare current retroviral vectors to SIN vectors, vectors with insulators, and lentiviral vectors. Regarding the potential for transgenes to be oncogenic, Dr. Copeland suggested checking to see if the transgene is in the RTCGD and to also consider homologs or genes in the same pathway. While animal models should be used to study the oncogenic potential of transgenes, no ideal assay exists. The

genetic background of the host is also presumably important since leukemias detected in different mouse strains were due to insertional mutagenesis of different genes.

The differences between the French and British X-SCID trials were discussed. The cell culture conditions and growth factors used varied between the trials. The vectors were essentially identical except for one base pair; however, the vectors were pseudotyped with different envelopes: the GALV envelope for the British trial and an amphotrophic envelope for the French. Since the population of CD34+ cells isolated for transduction was probably heterogeneous, there was the possibility that the differently pseudotyped vectors preferentially transduced different cell types which could have different propensities for generating leukemia or possibly influence integration sites. The differences between the research participants with leukemia and the others in the trial are also being studied. There were no striking differences in insertion sites. The transcription profiles of the participants' cells are being studied to determine if there was a higher propensity for cell proliferation in the participants whose cells expanded the most during culture allowing them to receive the higher doses.

Discussants agreed that the third leukemia patient from the French X-SCID study represents an opportunity to think about how monitoring should be conducted. In mid-November 2004, this individual went through the annual follow-up, including LAM-PCR, with normal clonality. In early January 2005 he developed overt disease. Because LAM-PCR is too expensive to do every month, clinical monitoring for malignant transformation by indicative symptoms and lab results should be conducted frequently.

Session III: BMT for SCID

Moderators: Theodore Friedmann, M.D., University of California, San Diego; Thomas Holohan, M.D., Office of Biotechnology Activities, NIH; and Bernard Lo, M.D., University of California, San Francisco

Overview of HLA Identical and Haploidentical BMT: The Duke University Medical Center Experience

Rebecca H. Buckley, M.D., Duke University Medical Center, provided an update on BMT at Duke University and summarized some of the other studies that have been published. SCID is caused by mutations in ten genes which encode cytokine or antigen receptors or other functions. The mutations result in different phenotypes. All SCID patients have no T cells, but some SCID patients have B cells, and some have NK cells. In all types of SCID, the thymus is present but small (less than 1 gram), corticomedullary distinction is lacking, and thymocytes and Hassall's corpuscles are absent.

The first BMT occurred in 1968. Up until the early 1980s, strict HLA identity was required between the donor and recipient to avoid host vs. graft disease (GVHD). One of the most important developments in the treatment of SCID in the past 22 years has been the use of T cell depletion which allows the mother or father to be the donor without the risk of GVHD.

From 1982 to 2005, 109 of 141 (77 percent) of patients treated at Duke are surviving. Survival time ranges from 1 month to 22.9 years posttransplantation. Of 16 HLA identical BMT patients at Duke, 100 percent are surviving; of 125 HLA haploidentical transplants, 93 (74 percent) are surviving. A survey conducted a few years ago showed the worldwide experience with haploidentical donors in treating children with SCID: 252 of 417 (60 percent) haploidentical transplants were successful. For all the different types of SCID, about half of those treated were surviving at the time of the worldwide survey. At Duke the patients died of pre-existing infections. Although 2.8% of these patients died of EBV lymphoproliferative disease, she was not aware of any cases of leukemia in SCID patients.

Factors influence the outcome of the transplants include the age of the recipient, the presence of infections, the genetic type of SCID and the match of the transplant. Experience at Duke and elsewhere indicates a high probability of survival for SCID patients who are transplanted within the first 3½ months after birth before infection. T-cell-depleted haploidentical BMT provides life-saving therapy for all forms of SCID, but it is not a perfect treatment. Initial success with gene transfer offers hope that the remaining defects could eventually be correctable by that means.

Overview of HLA Identical and Haploidentical BMT: The Memorial Sloan-Kettering Cancer Center Experience

Richard J. O'Reilly, M.D., Memorial Sloan-Kettering Cancer Center, presented results based on studies of T-cell-depleted transplants for SCID.

Dr. R. O'Reilly reported on a review of 118 cases of transplants for SCID conducted between 1980 and 1995. These cases included a variety of genetically disparate SCID types, although a large proportion of these patients were not genetically defined. Durable engraftment was achieved in 96 of these 118 individuals (81 percent): 75 percent experienced full reconstitution of their T cells, 25 percent had partial reconstitution, and a smaller fraction developed B-cell reconstitution. Seven of these patients developed grade 1 or 2 GVHD, but no grade 3 or 4 GVHD was seen.

Studies of long-term results of patients with 10 to 15 years of followup show that, for individuals who had NK cells before transplantation, the long-term disease-free survival without conditioning was 41 percent, but with conditioning, survival was 77 percent. In the group without NK cells prior to transplantation, survival without conditioning is about 76 percent, but with conditioning there is a 91 percent long-term disease-free survival. Thus, these studies indicate that conditioning for patients with ADA-SCID or for those with high levels of NK cells might be associated with a significant improvement in long-term survival.

Studies of patients who did not receive cytoreduction show that engraftment and reconstitution of the T-cell function were achieved, but only a small number of these patients showed evidence of B-cell reconstitution. In contrast, patients who received either busulfan or thiotepa in the pregrafting period consistently showed full or partial reconstitution of T-cell function as well as consistent engraftment of B cells and full reconstitution of B-cell function.

Dr. R. O'Reilly summarized the Memorial-Sloan Kettering experience with 68 SCID patients. Although 20 of those patients have died, 48 are long-term disease-free survivors. In the noncytoreduced population, lingering deficiencies in CD4 cells are seen, and at least half of these patients do not reconstitute NK-cell function in the postgrafting period; in contrast, all those who were cytoreduced are in the normal range in terms of NK-cell numbers and NK-cell function. Data from these patients also suggest that engraftment with donor B cells will result in functional reconstitution of the antibody system. Among the patients who did not receive cytoreduction, a significant drop in T-cell number and function was observed. Another hypothesis is that it may be important to engraft the early progenitor cells. In the cytoreduced patients, 15 to 20 years out, few late effects are being observed; however, in the noncytoreduced group, many of the patients exhibit interstitial nephritis, hepatitis, chronic obstructive lung disease, and warts. Without cytoreduction, Memorial-Sloan Kettering's X-SCID patients in the later followup years have no B-cell function, only 3 of 14 have NK-cell function, and all of them achieve T-cell function; however, a late decline is evident in nearly half. These data stand in contrast to the cytoreduced X-SCID patients, who are basically fine in terms of B-cell, T-cell, and NK-cell function.

Engraftment of donor NK cells is important, and engraftment of donor B cells is critical for reconstitution of humoral immunity. Maintenance of an adequate pool of thymic precursors also may be important to sustain the thymus over many decades. In addition, maintenance of thymic constituents may be critical for positive selection of antigen-reactive cells, as well as for negative selection against autoreactive T cells.

Overview of Umbilical Cord Blood (UCB) Transplantation

John E. Wagner, M.D, University of Minnesota, provided an overview of what is currently known about UCB transplantation and shared data from the experience of the New York Blood Center with SCID and Wiskott-Aldrich syndrome (WAS).

The literature indicates a range of 50 percent to 77 percent of SCID patients as surviving with unrelated BMT with complete immune reconstitution reported in a majority of the cases. However unrelated BMT is limited by donor availability and includes the potential for late effects such as chronic GVHD, infertility, and growth failure. Other limitations of unrelated donor BMT include adverse effect of HLA mismatch, a prolonged interval between search initiation and donor acquisition, high risk of acute and chronic GVHD, and high risk of opportunistic infection.

UCB is an alternative source of hematopoietic stem cells. There are some differences in the immune aspects of UCB as compared to adult stem cell sources. These immune properties of UCB include lower cytotoxicity, increased suppressor-cell activity, an altered T-cell cytokine production profile, and tolerance to the noninherited maternal allele, leading to less HLA restriction, no requirement for T-cell depletion, and reduced GVHD. Due to these properties, UCB may extend the donor pool, allowing greater access to hemopoietic SCT. Currently an adequately matched UCB donor can be found for almost all patients in approximately three hours. At the University of Minnesota, engraftment occurred in 88% of patients receiving UCB, a similar level to that achieved with BMT. Survival was influenced by HLA mismatch and cell dose. Higher cell doses were found to partially overcome the barrier of HLA mismatch.

Regarding UCB transplantation in the treatment of immunodeficiency disorders, critical issues include that most patients are young, many patients come to transplant with infections, and most do not have HLA-identical sibling donors and are therefore forced to undergo haploidentical or unrelated-donor transplantation.

Dr. Wagner summarized data available on 52 of the 57 SCID patients transplanted with UCB from 1995 to 2004 at the New York Blood Center. The median age was 11 months, 59 percent were non-Caucasian, most received a mismatch transplant with high cell doses and some immunosuppressive therapy to improve engraftment, and the overall incidence of neutrophil recovery was 81 percent. Despite the high incidence of engraftment, that percentage is not as high as that usually seen with leukemia patients, so some work remains to be done to improve patient outcomes. The overall incidence of grades 2 to 4 GVHD was 24 percent, and the overall survival rate was 57 percent.

Data on 33 WAS patients at the New York Blood Center show a median age of 19 months, the majority receiving mismatched transplants, and a cell dose that was considerably higher than what was observed for most of the Center's leukemia patients. The majority of these patients received busulfan and Cytoxan. The overall engraftment rate was higher than what was observed with the SCID patients, the overall incidence of GVHD was 26 percent, and the overall survival rate was 67 percent.

Conclusions for the New York Blood Center's SCID and WAS patients are that engraftment has been suboptimal for patients with SCID despite high cell dose, no data are available on the extent of immune reconstitution, and survival rates are comparable to results obtained with HLA-matched, unrelated-donor marrow.

In the pediatric population, there has been a tremendous increase in the use of UCB in recent years, such that the use of UCB has now surpassed the use of unrelated bone marrow. In the not-too-distant future, the adult population also may benefit from UCB transplants. The next generation of unrelated-donor UCB transplantation will focus on improving engraftment, and reducing transplant-related mortality and late effects.

Discussion

The discussion session focused on defining a population that would be candidates for gene transfer. Dr. Buckley considered a patient who did not become immune reconstituted following T cell depleted haplotransplant from the mother and father to be a candidate for gene transfer. Dr. O'Reilly suggested that the decision would depend on the clinical condition of the patient. If after a failing transplant, the patient was still in good enough clinical condition to withstand a graft with cytoreduction, he would recommend that approach. If however, the patient is *in extremis*, gene transfer would be appropriate.

Noting the success in treating patients who receive medical attention very early in life prior to becoming infected, Dr. Puck suggested that patients who responded poorly to transplant should be considered for gene transfer. Dr. Malech noted that for both transplantation and gene transfer efficacy dropped with older patients, so age should be considered in treatment decisions. Dr. Wagner agreed that gene transfer should be considered after failed transplantation unless specific risk factors could be identified that might indicate a patient should have gene transfer rather than a transplant. Dr. Buckley suggested that if the risks to gene transfer could be lowered, another indication for gene transfer would be for patients who are T cell chimeras without B cell and NK cell function.

Given the success of treatment of uninfected newborns, the RAC discussed the utility of neonatal screening. There are no other clinical indications that a child has SCID until they develop infections. However, methods are available to screen for SCID through lymphocyte counts or a DNA based assay for TRECs. While SCID is rare disease, effective therapy is available; therefore, the feasibility of newborn screening for SCID should be studied.

RAC Recommendations and Conclusions

On December 5, 2002, February 10, 2003, and March 15, 2005, the NIH Recombinant DNA Advisory Committee (RAC) reviewed the clinical and molecular data concerning three adverse events that occurred in a human gene transfer study being conducted in France to correct X-linked SCID. This study involves engraftment of an autologous bone marrow derived, $CD34^+$ hematopoietic stem cell enriched, cell population transduced with a Moloney murine leukemia retrovirus derived replication incompetent vector encoding the common gamma chain (γc) transmembrane protein subunit shared by receptors for Interleukins 2, 4, 7, 9, 15 and 21. Three children in this study developed T-cell acute lymphoblastic leukemia (T-ALL) almost 3 years after their gene therapy treatment. The leukemias appear to share the common causative mechanism of insertional mutagenesis at or near oncogenes. In the first two participants, the vector inserted at or near the LMO-2 gene with aberrant production of lmo-2 protein, which contributed to the abnormal growth of the leukemic cells. The integration sites in the cells of the third participant appear to involve LMO-2, and three other oncogenes (Science 308: 1735-1736). The unregulated expression of the γc transgene in the vector may also have a cooperative role in the induction of oncogenesis. An analysis of the available data from this and other gene transfer clinical trials for SCID led the NIH RAC to conclude that:

- The majority of children in this X-linked SCID gene transfer study have had major clinical improvement to date.

- Of the nine children in this experimental study who had successful engraftment of their gamma-c (γc) transduced cells, three developed leukemia approximately 3 years after treatment and have required chemotherapy; one participant subsequently died. The overall frequency of this adverse event in this trial cannot be determined at this time.

- The gene transfer was a cause of the leukemias.

- The occurrence of leukemia in this protocol is not a random event and constitutes a serious inherent risk in this study.

- Some subjects in gene transfer studies for non-X-linked SCID experienced mild to moderate clinical improvement.

These findings led the NIH RAC to make the following recommendations, which will be reviewed and potentially revised as new data become available.

- Retroviral gene transfer studies for X-linked SCID should be reviewed, on a case-by-case basis, and limited, pending further data, to patients who have failed identical or haploidentical stem-cell transplantation or for whom no suitable stem cell donor can be identified. Case-by-case review would

include appropriate risk:benefit analysis accompanied by implementation of appropriate informed consent and monitoring plans.

- There are not sufficient data or reports of adverse events directly attributable to the use of retroviral vectors at this time to warrant cessation of other retroviral human gene transfer studies, including studies for non-X-linked SCID. Such studies may be justified contingent upon appropriate risk:benefit analysis accompanied by implementation of appropriate informed consent and monitoring plans.

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